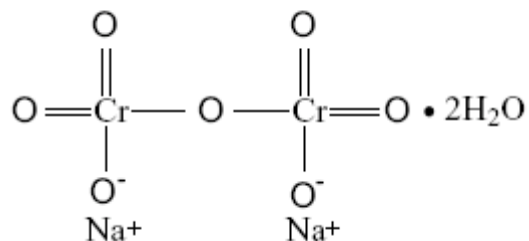




NTP
National Toxicology Program

Drinking Water Studies of Sodium Dichromate Dihydrate



Michelle J. Hooth





Nomination History

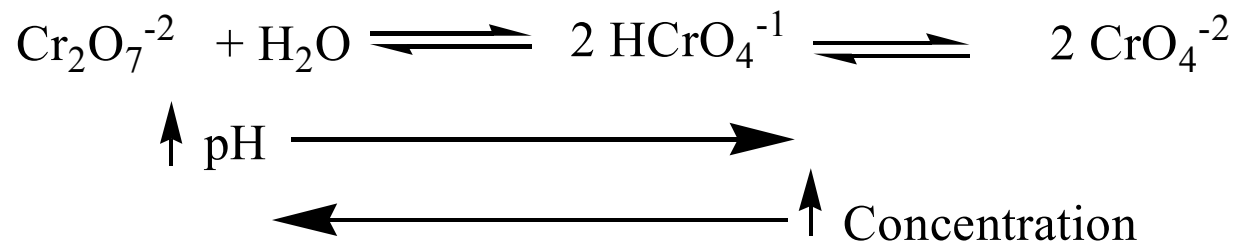
- Hexavalent chromium (VI) nominated for toxicity and carcinogenicity studies
 - CA Congressional Delegation, CalEPA, CA Department of Health Services
 - Cr(VI) detected in many CA drinking water sources
 - Drinking water contaminant from industrial processes: electroplating, leather tanning, textile manufacturing
 - Human carcinogen by the inhalation route
 - Lack of carcinogenicity studies of ingested Cr VI



Selection of Sodium Dichromate Dihydrate

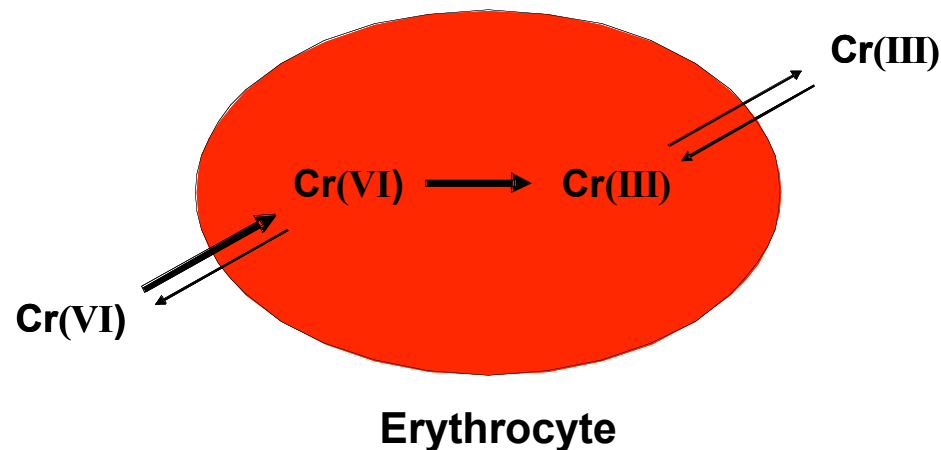
- Primary base material for the production of chromium compounds
- Widely used in industrial applications
- Most water soluble chromate salt
- Dichromate and chromate are in equilibrium in aqueous solution
- Form of Cr(VI) present depends on pH and concentration

Dichromate/Chromate Equilibrium





Hexavalent Chromium vs Trivalent Chromium



- Cr(III) is an essential nutrient and is relatively nontoxic
- Cr(VI) is poorly absorbed when ingested
- Rapid extracellular reduction of Cr(VI) to Cr(III) in acidic environments
- Greater absorption of Cr(VI) than Cr(III) because it readily enters cells by facilitated diffusion via anion channels in contrast to Cr(III) which enters by passive diffusion or phagocytosis
- Intracellular reduction of Cr(VI) to Cr(III) by ascorbate and GSH



Comparative Sodium Dichromate Dihydrate Absorption Study

- Selection of test species for NTP studies
- Male F344/N rats, B6C3F1 mice, and Hartley guinea pigs
- Similar to humans, guinea pigs do not have a forestomach and require a reducing agent in their diet
- Six sodium dichromate dihydrate drinking water concentrations (1-300 mg/L) for 21 days
- Total chromium measured in blood and kidney
 - Chromium concentrations increased with exposure concentration
- Rat and mice considered to be appropriate test species



Results of 3-Month Studies in Rats and Mice

- Drinking Water Concentrations: 0, 62.5, 125, 250, 500, or 1,000 mg/L
- Survival was similar to controls
- Mean body weights and body weight gains less than controls
 - Male rats: 500 and 1,000 mg/L
 - Female rats: 1,000 mg/L
 - Male and female mice: ≥ 125 mg/L
- Water consumption less than controls
 - Male and female rats: ≥ 250 mg/L
 - Male and female mice: ≥ 125 mg/L
- Hematology
 - Microcytic hypochromic anemia in rats and mice



Nonneoplastic Lesions in Rats and Mice

- Focal ulceration, hyperplasia, and metaplasia in the glandular stomach of male and female rats exposed to 1,000 mg/L
- Epithelial hyperplasia in the duodenum of male and female mice
- Histiocytic cellular infiltration
 - duodenum of rats and mice
 - liver of female rats
 - pancreatic lymph nodes of rats
 - mesenteric lymph nodes of mice
- Similar effects observed in other strains of mice



Genetic Toxicology Studies

- Mutagenic in *Salmonella* strains TA100 and TA98 (+/- S9)
- Mutagenic in *E. Coli* strain WP2 (+/- S9)
- Micronucleus tests:
 - Negative in male and female B6C3F1 mice
 - Equivocal in male B6C3F1 mice
 - Negative in male BALB/c mice
 - Positive in male *am3*-C57BL/6 mice



Design of 2-Year Studies in Rats and Mice



Agenda & Staff Presentations

Design Review *Hexavalent Chromium*

July 24, 2002

Updated: July 29, 2002

National Institute of Environmental Health Sciences (NIEHS)
Rodbell Conference Center, Rall Building
111 T.W. Alexander Drive
Research Triangle Park, North Carolina

Exposure Concentration Selection:

Male and female rats, female mice: 0, 14.3, 67.3, 172, or 516 mg/L

Male mice: 0, 14.3, 28.6, 85.7, or 257.4 mg/L



Results of 2-Year Studies in Rats

- Survival was similar to controls
- Mean body weights were less than controls in males and females exposed to 516 mg/L
- Water consumption was less than controls in males and females exposed to 172 and 516 mg/L
- Transient microcytic hypochromic anemia observed



Neoplastic Lesions in the Oral Cavity of Rats

Lesion	Sex	Concentration (mg/L)				
		0	14.3	57.3	172	516
Oral Mucosa						
Squamous Cell Papilloma	M	0	0	0	0	1
	F	0	0	0	0	0
Squamous Cell Carcinoma	M	0	0	0	0	6*
	F	0	0	0	2	11**
Tongue						
Squamous Cell Papilloma	M	0	0	0	0	1
	F	1	1	0	0	0
Squamous Cell Carcinoma	M	0	1	0	0	0
	F	0	0	0	1	0
Combined	M	0	1	0	0	7**
	F	1	1	0	2	11**

N=49-50; *P≤0.05; **P≤0.01



Histiocytic Cellular Infiltration in Rats

Lesion	Sex	Concentration (mg/L)				
		0	14.3	57.3	172	516
Liver	M	1	0	2	5	34**
	F	1	5	21**	42**	47**
Duodenum	M	0	0	6*	36**	47**
	F	0	0	1	30**	47**
Mesenteric Lymph Node	M	13	11	30**	39**	41**
	F	21	18	27	36**	42**
Pancreatic Lymph Node ^a	F	17	20	23	32**	27

N=46-50; ^aN=31-38

*P≤0.05; **P≤0.01



Results of 2-Year Studies in Mice

- Survival was similar to controls
- Mean body weights were less than controls in females exposed to 172 and 516 mg/L
- Water consumption was less than controls in males exposed to 85.7 and 257.4 mg/L and females exposed to 172 and 516 mg/L
- Transient microcytosis observed



Neoplastic Lesions in the Small Intestine of Male Mice

Lesion	Concentration (mg/L)				
	0	14.3	28.6	85.7	257.4
Duodenum					
Adenoma	1	0	1	5	15**
Carcinoma	0	0	0	2	3
Jejunum					
Adenoma	0	0	0	0	3
Carcinoma	0	2	0	1	2
Duodenum, Jejunum, or Ileum					
Adenoma or Carcinoma	1	3	2	7*	20**

N=50; *P≤0.05; **P≤0.01



Neoplastic Lesions in the Small Intestine of Female Mice

Lesion	Concentration (mg/L)				
	0	14.3	57.3	172	516
Duodenum					
Adenoma	0	0	2	13**	12**
Carcinoma	0	0	0	1	6*
Jejunum					
Adenoma	0	1	0	2	5*
Carcinoma	1	0	2	2	1
Duodenum, Jejunum, or Ileum					
Adenoma or Carcinoma	1	1	4	17**	22**

N=50; *P≤0.05; **P≤0.01



Nonneoplastic Lesions in the Small Intestine of Mice

Lesion		Concentration (mg/L)				
Males		0	14.3	28.6	85.7	257.4
Duodenum						
Focal Epithelial Hyperplasia	0	0	0	1	2	
Diffuse Epithelial Hyperplasia	0	11**	18**	42**	32**	
Females		0	14.3	57.3	172	516
Duodenum						
Focal Epithelial Hyperplasia	0	0	1	2	0	
Diffuse Epithelial Hyperplasia	0	16**	35**	31**	42**	
Jejunum						
Diffuse Epithelial Hyperplasia	0	2	1	0	8**	

N=50; **P≤0.01



Histiocytic Cellular Infiltration in Mice

Lesion		Concentration (mg/L)				
Males		0	14.3	28.6	85.7	257.4
Duodenum	0	2	4	37**	35**	
Mesenteric Lymph Node	14	38**	31**	32**	42**	
Pancreatic Lymph Node ^a	0	2	2	5*	12**	
Females		0	14.3	57.3	172	516
Duodenum	0	0	4	33**	40**	
Jejunum	0	0	0	2	8**	
Mesenteric Lymph Node	3	29**	26**	40**	42**	
Pancreatic Lymph Node ^a	0	1	2	7**	8**	
Liver	2	15**	23**	32**	45**	

N=46-50; ^aN=12-21

*P≤0.05; **P ≤0.01

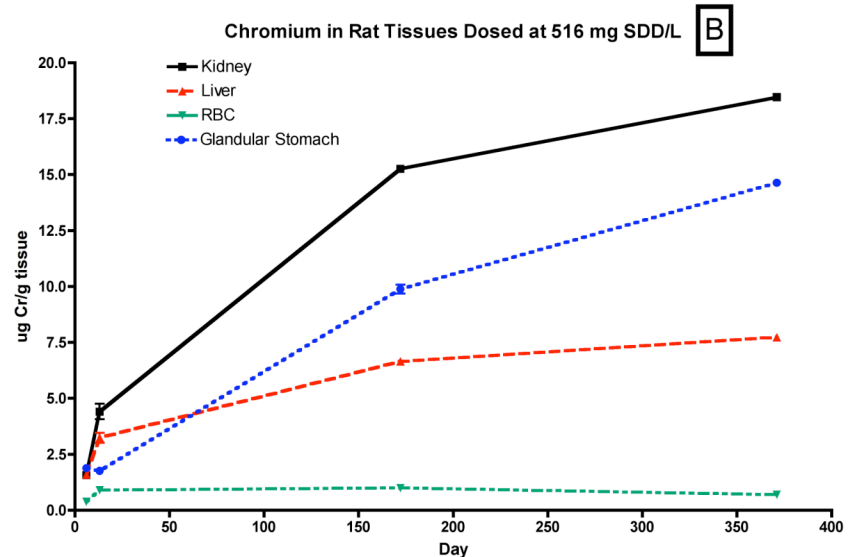
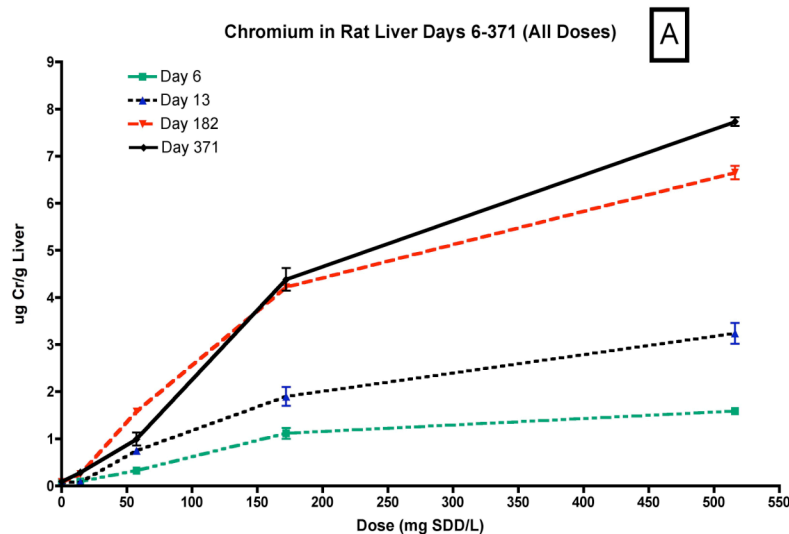


Chromium Tissue Distribution

- Additional groups of male F344/N rats and female B6C3F1 mice
 - Treated the same as core study animals
- Total chromium concentrations determined in selected tissues and excreta at multiple time points
 - Erythrocytes, plasma, liver, kidney, glandular stomach, forestomach
 - Days 6, 13, 182, and 371
- 48-hour washout period to allow excretion of unabsorbed Cr



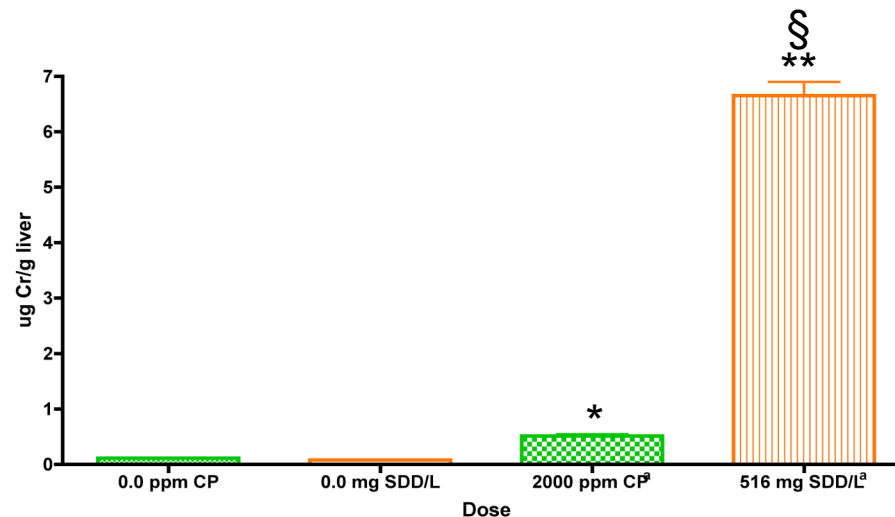
Chromium Concentrations in Tissues of Male Rats



- In general, Cr concentrations tended to increase with increasing exposure concentration and duration of exposure
- [Cr] was not proportional to exposure concentration indicating saturation of active uptake mechanism for Cr(VI)
- [Cr] was not proportional to duration of exposure indicating an equilibrium between Cr(VI) uptake and Cr(III) diffusion from the tissues and from cell turnover
- Highest Cr concentrations were in the kidney, glandular stomach, and liver



Comparison of Cr Concentrations in Male Rat Tissues Following Oral Administration as Cr(III) or Cr(VI)



- The NTP conducted two 2-year studies of chromium
 - Cr(III)- chromium picolinate monohydrate in feed
 - Cr(VI)- sodium dichromate dihydrate in drinking water
- Exposure concentrations were converted to mg Cr/kg bw using the food and water consumption data for weeks 1-25 of each study
 - Cr(III)- low concentration was 2,000 ppm and 15.2 mg Cr/kg bw
 - Cr(VI)- comparable concentration was 516 mg/L and 8.95 mg Cr/kg bw
- Increased Cr tissue concentrations when administered as Cr(VI)



Conclusions of the 2-Year Studies

- There was *clear evidence of carcinogenic activity* of sodium dichromate dihydrate in male and female F344/N rats based on increased incidences of squamous cell neoplasms of the oral cavity.
- There was *clear evidence of carcinogenic activity* of sodium dichromate dihydrate in male and female B6C3F1 mice based on increased incidences of neoplasms of the small intestine.
- Exposure to sodium dichromate dihydrate resulted in histiocytic cellular infiltration in multiple tissues of rats and mice and diffuse epithelial hyperplasia in the small intestine of male and female mice.
- There was systemic exposure to sodium dichromate dihydrate based on the tissue distribution data.